Human Genome Sciences, Inc.
Lab Notebook #

Department Protein Expression
Subject IV
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National Brand
Computation Notebook
11 3/4" x 9 1/4", 4 x 4 Quad., 75 Sheets 43-648

0 73333 43648 8

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Ruben EXHIBIT #55

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Ruben EXHIBIT 2055
Ruben v. Wiley et al.
Interference No. 105,077
RX 2055

12.1.95 Transfection of CHO-dhfr⁻ cells with:

1) BMP (N346/BMP)
2) TNFβ (N346/TNFβ)
3) TNFβ (CHOI/TNFβ)

- 11.28.95 CHO dhfr⁻ cells confluent 75 flasks were diluted 1:10 and seeded into the wells of a 6 well dish
  (- resuspend cells of T75 in 10ml Dissoculture medium, seed 1ml in each well of the 6 well plate)
- incubate cells over night → 90-95% confluent
- 11.29.95 - remove the medium of the 6 wells
  - wash cells with PBS
  - add 1 ml transfect. Medium 1: MEM L⁺, 0% dFBS, 1% PS
- prepare lipofectin-Mix in 96 well plate:

A: 90 µl transfect. Medium 1 (6 wells)
10 µl Lipofectin

B: 50 µl transfect Medium 1
5 µg Expressionvector (N346/BMP, N346/TNF-β, CHO-I/TNFβ)
2 wells for each construct
1 µg N18

- mix A + B and incubate 45' at RT
- add transfectionmix dropwise to the cells in the 6 well
- incubate for 4-6 h at 37°C, 5% CO_2
- add 1ml transfect Med2 to each well of the 6 well dish: MEM L⁺, 10% dFBS, 1% PS

– 12.1.95 seed the cells in selection medium

2 wells of a 6 well dish were transfected with one of the expressionvectors

A) → seed cells of 1 well in selective medium containing 20 nM MTX

B) → seed the cells of the second well transfected with the same expressionvector in selective medium containing 100 nM MTX

Prepare selective medium:

– thaw 1ml of 5 mM MTX stock
– dilute 1:50, 1:100 in MEM2 – medium
⇒ [MTX] = 1 µM

– thaw 3ml of G418 (100 mg/ml)

	Selective Med. A: 20 nM MTX	Med B: 100 nM MTX
MEM2:	150 ml	150 ml
dFBS:	7.5 ml	7.5 ml
G418 (100 mg/ml):	1.5 ml	1.5 ml
PS:	1.5 ml	1.5 ml
1 µM MTX:	3 ml	15 ml

– remove the medium of the transfected cells in the 6 well dishes
– wash cells with PBS
– trypsinize cells with sterile Trypsin/EDTA 5'

- resuspend cells of each well in 45 ml selective medium
- seed cells of each well in 3 hybridoma-dishes, 15 ml each hybridoma plate
- incubate cells for 2 weeks

12.7.95 Freezing of the CHO-clones ST 10.1 (cells were transfected with the new expression vector CHO I), VIGF5 (5 µM MTX) old vector N346, VIGF2 (100 µM MTX), VIGF3 (100 µM MTX)

ST 10.1: cells of 5 85-90% confluent T225 flasks were frozen in 50 1 ml aliquots
1 × 10^6 cells / aliquot

VIGF5 (5 µM MTX): cells of 1 85-90% confluent T225 flask was frozen in 10 aliquots of 1 ml
1 × 10^6 cells / aliquot

VIGF2 (100 µM MTX): cells of 1 85-90% confluent T225 flask was frozen in 10 aliquots of 1 ml
1 × 10^6 cells / aliquot

VIGF3 (100 µM MTX): cells of 1 85-90% confluent T225 flask was frozen in 10 aliquots of 1 ml
1 × 10^6 cells / aliquot

- remove the medium of the T-flasks and wash the cells with 10 ml PBS
(3-4 T225 flasks can be handled in parallel)
- add 5 ml Trypsin / EDTA and incubate 5' at 37°C
- add 6 ml MEM2-Medium + 10% dFBS and resuspend cells in a final volume

Stably transfected CHO cells - CHO / TNF γ

1.3.95

Cells were transfected at 12.1.95 (see P.20) and seeded into Hybridoma plates for selection (see p.21)

Observations:

- for all 3 constructs there were no clones found when cells were seeded in medium that contained 100nM MTX
- BUP/N346 = 20nM MTX: on three hybridoma plates there could only 1 clone be found

(– clone was not transfered)

	Hybridoma plate #	Clones #
– TNFγ (N346):	1	2
	2	18
	3	6
– TNFγ (CHO)	1	10
	2	9
	3	11

- the hybridoma cells with clones were labeled with a marker
- tissueculture medium was removed and the plate was washed with 10ml PBS

Remaining PBS was removed out of the wells that contained clones, using a 20µl Pipet

- [redacted]
- 7 µl of Trypsin was added to the wells that contained clones and cells were incubated for 5' at 37°C
- cells of each well were transferred into the well of a 24 well diss

(into MEM α, 5% dFBS, L-PS, 20mM HTX/well)

1996 - Observation of growth of cells in 24 well dishes

TNFα	1.1	single cells	2.1	—
	1.2	transfer cells into 6 well diss	2.2	—
CHO	1.3	→ 6 well diss	2.3	→ 6 well diss
20mM HTX	1.4	—	2.4	—
	1.5	S.C.	2.5	—
	1.6	→ 6 well diss	2.6	—
	1.7	→ 6 well diss	2.7	→ transfer cells to T25
	1.8	→ 6 well diss	2.8	S.C
	1.9	→ 6 well diss	2.9	S.C
	1.10	S.C.		
	3.1	—		
	3.2	—		
	3.3	S.C		
	3.4	—		
	3.5	—		
	3.6	—		
	3.7	—		
	3.8	—		
	3.9	—		
	3.10	—		
	3.11	—		

TNF γ	1.1	S.C	2.6	–
L346	1.2	S.C	2.7	–
2mM MTX	~~1.3~~		2.8	–
	~~1.4~~		2.9	–
	2.1	–	2.10	–
	2.2	S.C	3.1	–
	2.3	S.C	3.2	–
	2.4	S.C	3.3	–
	2.5	–	3.4	–
	3.5	–		
	3.6	–		

17.9.96

CHO/DG44 cells : 1 vial was thawed and seeded into a T75 flask in MEM α - Medium, 5% dFBS, 1% PS

CHO/DG44 01.09.96 - 4

VIGF3 (100 µM) : 1 vial was thawed and seeded into a T75 flask in MEM α - Medium, 5% dFBS, 1% PS, 50 µM MTX

VIGF3 01.09.96 - 0

VIGFS (5 µM) : 1 vial was thawed and seeded into a T75 flask in MEM α - Medium, 5% dFBS, 1% PS, 5 µM MTX

VIGFS 01.09.96 - 0

CHO/DG44 cells : passage cells 1:4 into one T75 flask → CHO/DG44 01.09.96 - 5 — 1.12.96

VIGF3 (100 µM) : passage cells 1:4 into 4 T75 flasks

VIGF3 01.09.96 - 1

VIGFS (5 µM) : passage cells 1:4 into one T75 flask

VIGFS 01.09.96 - 1

Markus work (Markus - sick)

1/15/96.

6:00PM - CHO/dhfr⁻ DG44 (T-75) 1-11-96-5 → CHO/dhfr⁻ DG44 (T-225) 1/15/96-6

{ all of culture pellet
47.5 ml MEM-α⁺ + 1% PS (1/15/96, dz)
2.5 ml HiFBS (cat. 16000-044, lot 45P4546, GIBCO) }

VIGFS T-75 1-9-96 (5μMMTX) → VIGFS (T-225, 5μMMTX) 1/15/96

{ all of culture pellet
50 ml MEM-α⁻ + 5% DiFBS + 1% PS
with 5μMMTX (1/15/96, dz) }
(5 ml of 50μM MTX stock.)

TNF 82.7 TAFr/CHO (T-25, 20nM) → TAFr/CHO (T-75, 20nM) 1/15/96

{ all of culture pellet
40 ml MEM-α⁻ + 5% DiFBS + 1% PS
with 20nM MTX (1/15/96, dz) }
(0.8 ml of 500nM MTX)

{ 50μMMTX { 0.1 ml 5mM MTX
10 ml MEM-α⁻ + 5% DiFBS + 1% PS
500nM MTX { 0.1 ml 50μMMTX
10 ml MEM-α⁻ + 5% DiFBS + 1% PS }

– 4 T 75 flasks VIGF3 010996-1 submitted to Yueli Zhang

- ST5, ST10 cells: Comparative Study : 1.16.96

- to compare expression level after several passages ST5 and ST10 cells were treated and seeded in MEM α, 5% dFBS, 1% PS, 80µM MTX :

ST5 - 011096 - P0
ST10 - 011696 - P0

TNFγ clones (cells transfected with CHO.01 vector)

cells from 6 well dish, passage into T25 :

TNFγ, CHO, 1.2
TNFγ, CHO 1.3

cells from 24 well dish, passage into 6 well dish:

TNFγ, CHO, 1.10
TNFγ, CHO, 2.3
TNFγ, CHO, 2.9

cells from 6 well dish passage into 6 well dish :

TNFγ, CHO 1.6
TNFγ, CHO, 1.7
TNFγ, CHO, 1.9

1.17.96 TNFγ - CHO cell lines transfected at 11.29.95 and transfered into selective medium at 12.1.95

- several more clones were picked from hybridoma plates into 24 well dishes:

TNFγ (cells were transfected with the CHO 1 vector)

1) TNFγ 4.1 / 112995 / P01 to TNFγ 4.4 / 112995 / P01
2) TNFγ 5.1 / 112995 / P01 to TNFγ 5.10 / 112995 / P01
3) TNFγ 6.1 / 112995 / P01 to TNFγ 6.10 / 112995 / P01

TNFγ (cells were transfected with the N346 vector)

4) TNFγ 4.1 / 112995 / P01 to TNFγ 4.10 / 112995 / P01
5) TNFγ 5.1 / 112995 / P01 to TNFγ 5.4 / 112995 / P01

- medium was removed from the hybridoma plates
- hybridoma plate was washed with 10 ml PBS
- PBS was removed
- the wells that were marked and numbered for harvest before, were cleared from PBS using a 20µl Pipetman and a white tipped sterile yellow tip
- 5 wells were trypsinized at one time (to control time of incub. with Trypsin)
- 5' incubation at 37°C
- 10 µl of medium was added to the cell cells were transfered and seeded into the well of a 24 well plate in MEM α, 5% dFBS (7% PS, 20 mM MTX (using a 2µl Pipetteman.

1.22.96 TNFγ-clones: Cells expanded in T25 flasks for amplification and freezing of backups (MEM α, 5% dFBS, 1% PS, +20 nM MTX)
(CHO: cells were transfected with CaBO1 derived vector)

1.) CHO/TNFγ 1.3/112995-P04 : 1 T25 confluent in 2 T25 flasks
2.) CHO/TNFγ 1.2/112995-P04 : 1 T25 confluent in 2 T25 flasks
3.) CHO/TNFγ 2.7/112995-P04 : 1 T75 confluent 1:10 in 1 T25 flask
1:10 in 1 T75 flask

4.) CHO/TNFγ 1.6/112995-P04 : 1-6W confluent in 2 T25 flasks
5.) CHO/TNFγ 1.7/112995-P04 : 1-6W confluent in 2 T25 flasks
6.) CHO/TNFγ 1.9/112995-P04 : 1-6W confluent in 2 T25 flasks

7.) CHO/TNFγ 2.3/112995-P03 : 1-6W confluent in 2 T25 flasks
8.) CHO/TNFγ 2.9/112995-P03 : 1-6W 40% confl. in 2 T25 flasks

Next steps with this clones:
- all clones split in 2 T25 flasks, one flask of each clone will be expanded into a T75 flask for freezing of 3x1 ml aliquots at 1×10^6 cells/ml. Cells of the second T25 flask are going to be seeded in 6 wells of a 6 well dish at 0, 20, 50, 100, 200, 500 nM MTX for amplification

1.23.96 Comparative Study ~~[illegible]~~ 346 STS and CHO ST 10.1 in MEM α, 5% dFBS, 1% PS, 80 μM MTX

- Friday 1.19.96

- STS 011696-P00 → P01 : 1-8% confluent T75 flask split 1:3 into 3 T75 flasks
- ST 10.1 011696-P00 → P01 : 1-80% confluent T75 flask split 1:3 into 3 T75 flasks
  - 3 x 1ml aliquot of both clones were frozen at -80°C

- Tuesday 1.23.96

- 175 flasks are ca. 90% confluent, 1 T75 flask of each cell line was given to Maria Collins for long term comparison in spinner flasks
- 3 x 1ml aliquots of both cell lines were frozen at -80°C

- cells of 1 ST5 and 1 ST10.1 T25 flask were passaged into 2 T25 flask at a split ratio of 1:3 ⇒P2

Establishing of stable CHO-TNFγ clones 1.25.96

(All clones were transfected with the CHO-01 derived vector)

Clones: 1) CHO (TNFγ 1.3 / 112995-P04 → P05 — 2 confluent T25 flasks
2) CHO (TNFγ 1.2 / 112995-P04 → P05 — 2 confluent T25 flasks
3) CHO (TNFγ 2.7 / 112995-P04 → P05 — 1 confluent T25 flask, 1 confluent T25 flask
4) CHO (TNFγ 1.7 / 112995-P04 → P05 — 2 confluent T25 flasks
5) CHO (TNFγ 1.9 / 112995-P04 → P05 — 2 confluent T25 flasks

All the clones:

first T25 flask: - take a 2 ml aliquot of the TCM and freeze it in a cryovial at -20°C for testing in a Western assay.

freezing: - trypsinise cells and resuspend in 16 ml final volume, seed 10 ml in (1:1.6) T75 flask (MEM1, 5% dFBS, 1% PS, 20mM MTX)
⇒ freeze these cells on Tuesday

- cells of T75 TNFγ 2.7, split 1:5 in T75

second T25 flask of every clone:

- trypsinise cells, and resuspend in 2 ml MEM1, 5% dFBS, 1% PS
- seed 1 ml in a T25 flask in MEM1 5% dFBS, 1% PS, 20mM MTX
next day replace medium with CHO-SFM-II + 1% PS
in order to use WE and supernatant samples for Western + Dan Padmanabh's assays.

duplicate: - resuspend remaining 1 ml in a final volume of 6 ml MEM1, 5% dFBS, 1% PS, 0 MTX

- distribute 1 ml cell suspension in each of a well of a 6 well plate
- add 0 / 50 µl / 150 µl / 250 µl / 500 µl and 1000 µl of MEM α, 5% dFBS, 1% PS, 800 mM MTX in order to achieve 0 / 20 mM / 60 mM / 100 mM, 200 mM and 400 mM MTX final concentrations in a final volume of 2 ml / well
- adjust final volumes to 2 ml / well with MEM α, 5% dFBS, 1% PS, 0 MTX.

T.26.96 **Comparative Study 346 STS and CHO ST 10.1:**

1) - take 3 x 1 ml samples of each clone:

346 STS / 011696 - P02
CHO ST10.1 / 011696 - P02

freeze samples at - 80°C

2) - passage cells 1 : 4 after WE. seed 2 flasks / clone
medium: MEM α, 5% dFBS, 1% PS
80 µM MTX.

→ 346 STS / 011696 - P03
CHO ST10.1 / 011696 - P03

CHO dhfr - / DG 44 cells

Passage:

CHO dhfr - DG 44 / 010996 - P08 → P09 1:10 seed 2 flasks

Medium: MEM α, 5% dFBS, 1% PS,

Establishing of stable CHO-TNFγ-clones

1.) 1) CHO TNFγ 2.3 / 112995-P03 → P04
2) CHO TNFγ 2.9 / 112995-P03 → P04
3) CHO TNFγ 1.6 / 112995-P03 → P04

- cells were confluent in 2 x T25 flasks each clone
- split cells 1:5 and seed in 2 T25 flasks each clone in MEMα, 5% dFBS, 1% PS, 20mM HEPES

2.) TNFγ clones growing in 6well dishes, passage and seed cells in 6well dish: MEMα, 5% dFBS, 1% PS, 20mM HEPES

1) CHO TNFγ 3.3 / 112995-P02-03
2) CHO TNFγ 1.10 / 112995-P02-03
3) CHO TNFγ 1.8 / 112995 medium was exchanged in a 6-well dish

3.) Clones in 24 well dishes, passage and seed cells in T25 or 6-well dish: MEMα, 5% dFBS, 1% PS, 20mM HEPES

passage in 6well dish:
1) CHO TNFγ 4.1 / 112995 P01 → 02
2) CHO TNFγ 4.2 / 112995 P01 → 02
3) CHO TNFγ 4.3 / 112995 P01 → 02
4) CHO TNFγ 4.4 / 112995 P01 → 02
5) CHO TNFγ 5.5 / 112995 P01 → 02
6) CHO TNFγ 5.7 / 112995 P01 → 02
7) CHO TNFγ 5.9 / 112995 P01 → 02
8) CHO TNFγ 6.2 / 112995 P01 → 02
9) CHO TNFγ 6.10 / 112995 P01 → 02
10) CHO TNFγ 6.6 / 112995 P01 → 02

passage in T25 dish:
1.) CHO TNFγ 6.4 / 112995 P01-02
2.) CHO TNFγ 6.7 / 112995 P01-02
3.) CHO TNFγ 6.8 / 112995 P01-02

4.) N396 clones of clones, cells grow in 24 well dishes, passage and seed cells in 6 T25 flasks in MEMα, 5% dFBS, 1% PS

1) 396 TNFγ 2.3 / 112995 P01-02
2) 396 TNFγ 4.1 / 112995 P01-02
3) 396 TNFγ 4.3 / 112995 P01-02
4) 396 TNFγ 4.4 / 112995 P01-02
5) 396 TNFγ 4.5 / 112995 P01-02
6) 396 TNFγ 4.6 / 112995 P01-02

7.) 346-TNFγ 4.7 / 112995-P01-02
8.) 346-TNFγ 4.10 / 112995-P01-02
9.) 346 TNFγ 5.3 / 112995-P01-02
10.) 346 TNFγ 5.4 / 112995-P01-02

1.27.96 Establishing of stable CHO-TNFγ-clones

Clones for testing in Western blots and in Dan Bednarik's assays:

1) CHO TNFγ 1.2 / 112995-P05
2) CHO TNFγ 1.3 / 112995-P05
3) CHO TNFγ 1.7 / 112995-P05
4) CHO TNFγ 1.9 / 112995-P05
5) CHO TNFγ 2.7 / 112995-P05

cells were seeded into 125 flasks on 1.25.96 (p. 45)

- Exchange the tissue culture medium
  - remove medium
  - wash twice with PBS to make sure to remove all traces of MTX
  - add 5ml CHO-S-SFM II + 1% PS and incubate for 2 more days

- as negative control for the assays also change the medium to CHO-S-SFM II + 1% PS for clone #

ST 10.1 / 011696-P03

1.29.96
- harvest supernatants of clones above and aliquot each sample in 5 × 1ml aliquots
  - freeze down 3 aliquots/clone for westerns
  - submitt 2 × 1ml aliquot/clone to Dan Bednarik for antiviral assays.

Establishing of Stable CHO-TNFγ clones 1.30.96

T25 flasks confluent: → split 50% → T75 freezing, 25% → T25 analysis
25% → 6 well amplification

(all clones transfected with transfected with the pCHO 1 (pC1) derived vector

1.) CHO TNFγ 1.6 / 112995-P04-P05
2.) CHO TNFγ 2.3 / 112995-P04-P05
3.) CHO TNFγ 2.9 / 112995-P04-P05
4.) CHO TNFγ 6.4 / 112995-P02-P03
5.) CHO TNFγ 6.7 / 112995-P02-P03
6.) CHO TNFγ 6.8 / 112995-P02-P03

all cell lines are confluent in T25 flask

- remove Medium from flask
- wash cells with PBS
- trypsinise 500 µl Trypsin 5' 37°C
- resuspend in 2 ml final volume MEM α, 5% dFBS, 1% PS, 0 MTX

Freezing

1.) Seed 1 ml of the cell susp. in a T75 flask in 20 ml MEM α, 5% dFBS, 1% PS, 20 nM MTX to expand cells for freezing of selected clones

Amplifie

2.) Seed 0.5 ml in 5 ml MEM α, 5% dFBS, 1% PS, 0 MTX into the first well of a 6 well plate, mix well and seed 1 ml of the cell suspension into each well of the 6 well plate
- add 0 / 50 µl / 150 µl / 250 µl / 500 µl / 1000 µl of MEM α, 5% dFBS, 1% PS 800 nM MTX
- adjust volume to 2 ml each well
⇒ MTX end concentrations 0, 20 nM, 60 nM, 100 nM 200 nM, 400 nM

Testing

3.) Seed 0.5 ml of cell suspension in 6 T25 flask in MEM α, 20 nM MTX, 5% dFBS, 1% PS
- Change Med when cells are confluent to CHO-S-SFMII + 1% PS

- incubate for 2 more days, then harvest medium for testing in Western + Antiviral assay

Establishing of stable CHO-TNFγ clones (pC1-constructs)

1.30.96 freezing of inprocess cells after clone selection in hybridoma plates, expanding of clones in 24 well, 6 well, T25 and T75 flasks in presence of 20nM MTX

(continued from P. 95)

1) CHOTNFγ 1.2/112995-P05-06
2) CHOTNFγ 1.3/112995-P05-06
3) CHOTNFγ 1.7/112995-P05-06
4) CHOTNFγ 1.9/112995-P05-06
5) CHOTNFγ 2.2/112995-P05-06 20nM MTX

- trypsinise cells and resuspend in 10ml
- Spin 10' at 800 rpm
- resuspend cells in 3ml freezing medium:
  MEM-, 5%-dFBS, 10%-DMSO
- freeze down 3 × 1ml aliquots of each clone
  store in Isopropanol freezing box at -80°C overnight
- transfer cells into the -140°C freezer

1.30.96 CHOTNFγ-clones: expanding cells for freezing:

Cells are confluent in wells of a 6 well dish:
passage cells into a T75 flask in
MEM-, 5%-dFBS, 1%-PS, 20nM MTX

1) CHOTNFγ 3.3/112995-P03-04
2) CHOTNFγ 4.6/112995-P03-04
3) CHOTNFγ 6.2/112995-P02-03
4) CHOTNFγ 6.6/112995-P02-03
5) CHOTNFγ 6.10/112995-P02-03

Comparative Study 346 STS and CHO ST 10.1 — 1.30.96

Take 3 x 1ml aliquots of each clone and passage the cells in MEM α, 5% dFBS, 1% PS, 80, 4 HT

Split ratio 1:3

1.) 346 STS / 011696-P03-04
2.) CHO ST10.1 / 011696-P03-04

CHO dhfr⁻ / DG44 cells — 1.30.96

Passage

CHO dhfr⁻ DG44 / 010996-P09-10 Split 1:10 seed 2 flasks

MEM α⁺, 5% dFBS, 1% PS

Establishing of stable CHO-TNFγ clones. — 1.31.96

Harvest of medium for testing in Western and in antiviral assays.

1.) CHO TNFγ 1.6 / 112995-P05
2.) CHO TNFγ 2.3 / 112995-P05
3.) CHO TNFγ 2.9 / 112995-P05
4.) CHO TNFγ 6.4 / 112995-P03
5.) CHO TNFγ 6.7 / 112995-P03
6.) CHO TNFγ 6.8 / 112995-P03

T25 flasks 90% confluent

- remove tc-medium
- wash 2x with PBS
- add 5 ml CHO-S-SFM + 1% PS
- incubate for 2 more days

1.31.96 Establishing of CHO TNF γ clones

Amplification: continued from p.45

cells confluent at 60 nM MTX, decreasing # of Rights [MTX]:

1.) CHO TNF γ 1.2 / 112996 - P05 → 0C
2.) CHO TNF γ 1.3 / 112996 - P05 → 0C
3.) CHO TNF γ 1.7 / 112996 - P05 → 0C
4.) CHO TNF γ 1.9 / 112996 - P05 → 0C
5.) CHO TNF γ 2.7 / 112996 - P05 → 0C

- remove TCM, wash with PBS 1 ml / well
- trypsinise 5', 500 µl Trypsin / EDTA the cells with ~~0~~, ~~50~~, 60, 100, 200, 400 nM MTX
- add 1 ml MEM α, 5% dFBS, 1% PS, 0 MTX to each of the four cells
- pool the cells of the 4 wells in 6 ml final volume
- seed the 6 ml cells up into 6 wells a la 6 w. dish at 1 ml / well
- add 0, 50, 150, 250, 500 and 1000 µl of MEM α⁻, 5% dFBS, 1% PS, 800 nM MTX to achieve 0, 20 nM, 60 nM, 100 nM, 200 nM, 400 nM [MTX].
- add MEM α⁻, 5% dFBS, 1% PS 0 MTX to a final volume of 2 ml / well of the 6 well dish.

1.31.96 Amplification of VIRF 5 up to 100 µM MTX continued from P. 43:

- several clones / well from 20 to 100 µM MTX
- remove medium, trypsinise 5' 200 µl / well, resusp cells in 6 ml final volume in MEM α⁻, 5% dFBS, 1% PS, 0 MTX

- Seed cells in 6 wells of a 6-w. dish, 1 ml/well
- add 0, 100 µl, 200 µl, 500 µl, 800 µl, 1000 µl of 200 µM MTX to 1 well/each 0, 10 µM, 20 µM, 50 µM, 80 µM and 100 µM MTX (MEM α, 5% dFBS, 1% PS, 200 µM MTX)
- expand wells to 2 ml final volume/well (MEM α, 5% dFBS, 1% PS, 0 MTX)

Establishing of stable CHO-TNFγ clones (pEI const.) 2.196.

freezing of in process cells after clonal selection and expanding of clones in T 75 flasks in presence of 20 nM MTX.

(continued from P. 49)

1.) CHO TNFγ 1.6 / 112995-p06→07
2.) CHO TNFγ 6.4 / 112995-p03→04
3.) CHO TNFγ 6.7 / 112995-p03→04
4.) CHO TNFγ 6.8 / 112995-p03→04 (20 nM MTX)

- trypsinize cells and resusp. in 10 ml
- spin 10' at 800 RPM
- resuspend in 3 ml freezing medium (MEM α, 5% dFBS, 10% DMSO)
- freeze down 3 x 1 ml aliquots of each clone
- freeze in Mr. Frosty freezing box at -80°C overnight
- transfer cells in -140° C freezer.

Establishing stable 346-TNFγ clones (pN346 constructs)

expanding of clones for freezing (backups)
clones growing in 24 well dishes (see p. 42)

1.) 346 TNFγ 4.2 / 112995-P01-02
2.) 346 TNFγ 4.8 / 112995-P01-02
3.) 346 TNFγ 4.9 / 112995-P01-02
4.) 346 TNFγ 5.1 / 112995-P01-02
5.) 346 TNFγ 5.2 / 112995-P01-02

- remove TCM, trypsinize 200 µl 5:
- resusp. in 2 ml MEM α, 5% dFBS 1% PS, 20 nM MTX
- seed in 6 well

2.1.96 Establishing stable 346 TNFγ clones (pN346 derived)

Several more clones were picked from hygromycin plates into 24 well dishes:

1.) 346 TNFγ 6.1 / 112995 - p01
2.) 346 TNFγ 6.2 / 112995 - p01
3.) 346 TNFγ 6.3 / 112995 - p01
4.) 346 TNFγ 6.4 / 112995 - p01
5.) 346 TNFγ 6.5 / 112995 - p01
6.) 346 TNFγ 6.6 / 112995 - p01
7.) 346 TNFγ 6.7 / 112995 - p01
8.) 346 TNFγ 6.8 / 112995 - p01
9.) 346 TNFγ 6.9 / 112995 - p01
10.) 346 TNFγ 6.10 / 112995 - p01

Steps see protocol p. 42.

Establishing stable CHO TNFγ clones (pCI derived)

Clones that were growing in 6 well dishes or 24 well dishes were passaged into 6 well dishes in MEM α, 5% dFBS, 20 nM MTX 1% PS (Cells are going to be expanded into T25 flasks for freezing of backup clones)

from 24 well:
1.) CHO TNFγ 5.1 / 112995 - p01-02
2.) CHO TNFγ 5.2 / 112995 - p01-02
3.) CHO TNFγ 5.3 / 112995 - p01-02
4.) CHO TNFγ 5.4 / 112995 - p01-02
5.) CHO TNFγ 5-6 / 112995 - p01-02
6.) CHO TNFγ 5.10 / 112995 - p01-02
7.) CHO TNFγ 6.1 / 112995 - p01-02
8.) CHO TNFγ 6.5 / 112995 - p01-02

from 6 well:
9.) CHO TNFγ 4.3 / 112995 - p02-03
10.) CHO TNFγ 5.7 / 112995 - p02-03

2.1.96

clones that were growing in 6 well dishes were passaged into T25 or T75 flasks in MEMα, 5% dFBS, 1% PS, 20 nM MTX (expansion cells in T75 flasks for freezing at early clones) 2.1.96

1.) CHO TNFγ 4.1 / 112995-p02-03 → T25
2.) CHO TNFγ 4.2 / 112995-p02-03 → T25
3.) CHO TNFγ 4.4 / 112995-p02-03 → T25
4.) CHO TNFγ 5.5 / 112995-p02-03 → T25
5.) CHO TNFγ 5.9 / 112995-p02-03 → T25

Establishing of stable CHO clones TNFγ (pCI constr.) 2.2.96

freezing of in process cells after clonal selection and expanding clones into T25 flasks in presence of 20 nM MTX.

(continued from p 45. and 49)

1.) CHOTNFγ 2.3 / 112995-p06
2.) CHOTNFγ 2.9 / 112995-p06
3) CHOTNFγ 3.3 / 112995-p05
4.) CHOTNFγ 6.2 / 112995-p04
5.) CHOTNFγ 6.6 / 112995-p04
6.) CHOTNFγ 6.10 / 112995-p04

- trypsinise cells and resusp. in 10 ml
- spin 10' at 800 rpm, resusp. cells in 3 ml freezing medium (MEMα, 5% dFBS, 10% DMSO)
- freeze down in 3 x 1 ml aliquot each store in Nalgene freezing box at -80°C over night
- transfer cells in -140° freezer.

2.1.96

22.96 Establishing of stable CHO TNFγ clones

Harvest of medium for testing in western- and antiviral assays (continued from p. 51)

- after 2 days of incubation in CHO-S-SFM-II + 1% PS the medium was harvested and frozen in 5 x 1 ml aliquots each clone at -20°C

1.) CHO TNFγ 1.6 / 112995 p 5
2.) CHO TNFγ 2.3 / 112995 p 5
3.) CHO TNFγ 2.9 / 112995 p 5
4.) CHO TNFγ 6.4 / 112995 p 3
5.) CHO TNFγ 6.7 / 112995 p 3
6.) CHO TNFγ 6.8 / 112995 p 3

22.96 Comparative Study CHO ST 10.1 / 346 ST 5

take 3 x 1ml aliquots each clone and passage the cells in MEM-, 5% dFBS, 1% PS, 80 µM MTX

1.) 346 ST5 / 011696 - p 04-05 split 1:4
2.) CHO ST 0.1 / 011696 - p 04-05 split 1:4

Passaging of CHO dhfr- / DG44 cells

CHO dhfr- DG44 / 010996 - p 11 split 1:10.

seed one T225 flask in MEM-, 5% dFBS, 1% PS

22.96

Establishing of stable CHO TNF δ clones — 2.5.96

Freezing of clones (backup), in process cells after clonal selection, and expanding in T25 flasks of 20 nM MTX

1.) CHO TNF δ 1.10 / 112995 - p 05
2.) CHO TNF δ 5.5 / 112995 - p 04
3.) CHO TNF δ 5.9 / 112995 - p 04

Comparative Study CHO ST10.1 - 346 STS — 2.5.96

Freezing of clones for further analysis

1.) 346 STS / 011696 - p 05 - 06
2.) CHO ST10.1 011696 - p 05 - 06

Passaging of CHO dhfr⁻ / DG44 — 2.6.96

CHO dhfr⁻ DG44 / 010996 - p 12 split 1:10

seed one T225 flask in MEM α^+, 5% dFBS, 1% PS

Comparative Study CHO ST10.1 - 346 STS — 2.6.96

take 3 × 1 ml aliquots each clone and passage the cells in MEM α^-, 5% dFBS, 1% PS, 80 nM MTX

1.) 346 STS / 011696 - p 05-06 split 1:3
2.) CHO ST10.1 011696 - p 05-06 split 1:3

2.6.06 Establishing of stable CHO TNFγ

Expanding of cells for freezing, passage cells from 6 wells to T75 flasks in 20nM MTX (= freezing backups only)

1.) CHO TNFγ 6.5 / 112995 - p02-03
2.) CHO TNFγ 4.3 / 112995 - p03-04
3.) CHO TNFγ 5.7 / 112995 - p03-04
4.) CHO TNFγ 5.4 / 112995 - p02-03

Expanding of cells for freezing, passage cells from T25 to T75 flasks in 20nM MTX (freezing backups only)

1.) CHOTNFγ 4.1 / 112995 - p03-04
2.) CHOTNFγ 4.2 / 112995 - p03-04
3.) CHOTNFγ 4.4 / 112995 - p03-04

Establishing of stable CHO TNFγ clones

Amplification: continued from page 52

cells confluent at 200nM and few cells at 400nM

1.) CHOTNFγ 1.2 / 112995-p06-07
2.) CHOTNFγ 1.3 / 112995-p06-07
3.) CHOTNFγ 1.7 / 112995-p06-07
4.) CHOTNFγ 1.9 / 112995-p06-07
5.) CHOTNFγ 2.7 / 112995-p06-07

- remove TCM cells with 1ml PBS/well
- trypsinise 5' 200µl Trypsin/EDTA/well (only 200nM and 400nM well)
- pool cells of 2 wells and resuspend in 6ml FV on MEM α, 5% dFBS, 1% PS, 0 MTX
- seed the cells into 6 wells of a new 6 well dish, 1ml/well

800 µl

- add 0, 100, 200, 500, and 1000 µl of ~~Trypsin~~ MEM, 5% dFBS, 1% PS, 2 µM MTX to the wells in order to achieve 0, 100 nM, 200 nM, 500 nM, 800 nM and 1 µM MTX final concentration
- add MEM 2, 5% dFBS, 1% PS, 0 MTX, adjust final volume to 2 ml / well.

Establishing of single CHO TNFR clones 2.6.96

Amplification: continued from p. 49

cells grown in 6 wells 20 - 400 nM MTX

1.)	CHO TNFR 1.6 / U2995 - P05-06	pools	100, 200, 400 nM
2.)	CHO TNFR 2.3 / U2995 - P05-06	pools	20, 60, 100, 200, 400 nM
3.)	CHO TNFR 2.9 / U2995 - P05-06	pools	60, 100, 200, 400 nM
4.)	CHO TNFR 6.4 / U2995 - P03-04	pools	200, 400 nM
5.)	CHO TNFR 6.7 / U2995 - P03-04	pools	100, 200, 400 nM
6.)	CHO TNFR 6.8 / U2995 - P03-04	pools	200, 400 nM

- remove TCM wash with 1 ml PBS / well
- trypsinize 5', 200 µl Trypsin EDTA / well (wells that are pooled only)
- pool cells and resusp. in 6 ml final volume in MEM, 5% dFBS, 1% PS 0 MTX
- seed cells in 6 wells of a 6 well dish 1 ml / well
- add 0, 50, 150, 250, 500, 1000 µl of MEM, 5% dFBS, 1% PS 0.8 µM MTX to achieve 0, 20, 60, 100, 200, 400 nM MTX final concentration
- add MEM, 5% dFBS, 1% PS, 0 MTX adjust final volume to 2 ml / well.

27.96 Establishing of CHO TNFγ clones

Submitted samples for testing in antiviral assays.

1.) CHOTNFγ 1.6 / 112995 p5
2.) CHOTNFγ 2.3 / 112995 p5
3.) CHOTNFγ 2.9 / 112995 p5
4.) CHOTNFγ 6.4 / 112995 p5
5.) CHOTNFγ 6.7 / 112995 p5
6.) CHOTNFγ 6.8 / 42995 p5

Samples that were harvested on 2.2.96 (p56), were submitted to Dan Bednarik for testing for antiviral activity.

2.8.96 Establishing of stable CHOTNFγ clones

preparation of samples for Western analysis:

- all clones were seeded into 25cm² T flasks for testing see p. 45 and p. 49
- after over night incubation the medium was replaced by CHO-S-SFM II + 2% Serum (dialysed)
- cells were incubated for 2 more days before supernatants were harvested and frozen at -20°C. see p. 48, p 56

Freezer Box1-1

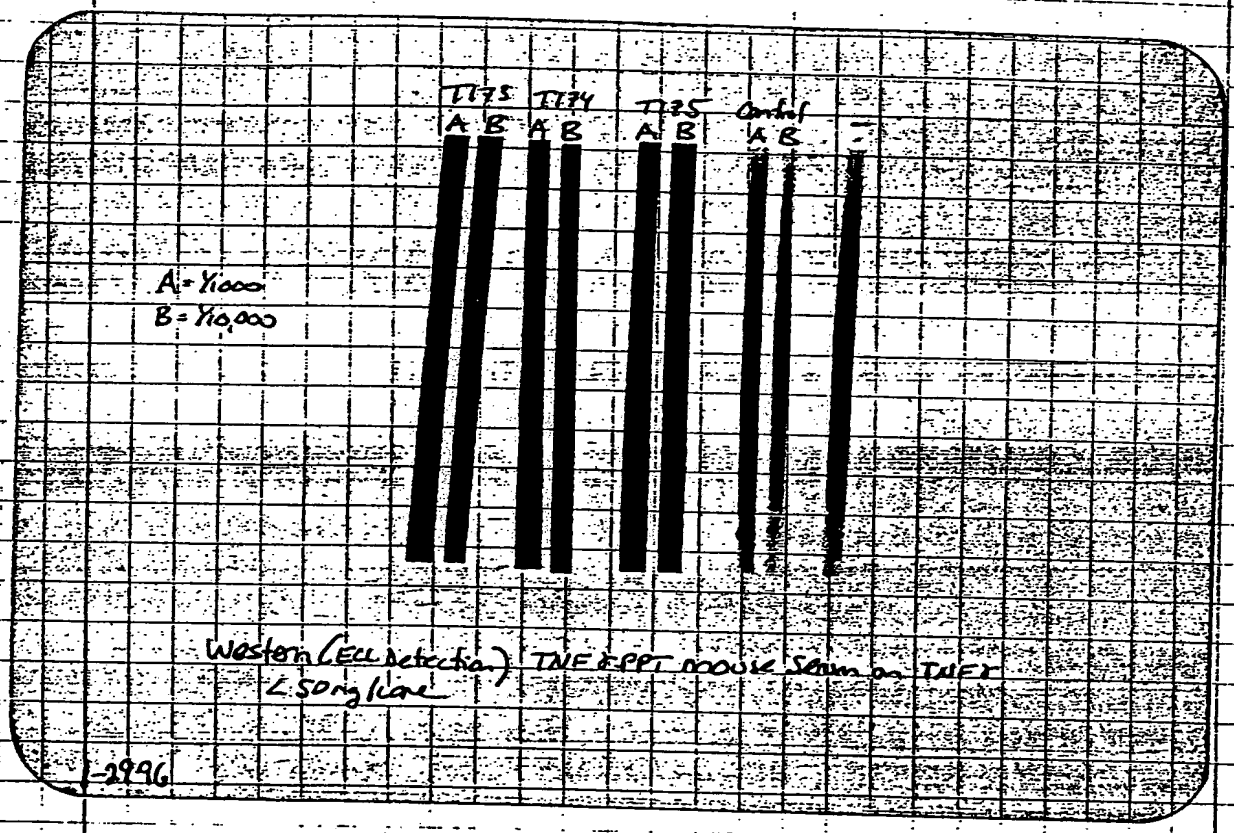
In Process Cells

Recombinant Cell Line:	CHO/pC1/TNFγ-IL6
Date:	02.07.96
Clone ID:	HLTBT71
Project Code:	HG10700
Rack Number:	1
Shelf Number:	1
Box Number:	1,1
Freezer:	Equipment Room/BPEC
Host Cell Line:	CHO dhfr- (ATCC)
Passage Number Host Cells:	-
Expression vector:	pC1/TNFγ-IL6
Construct made by:	Guo-Liang Yu
Date of Transfection:	11.29.95
Cell Line Established by:	Markus Buergin
Notebook:	443

	Clone Number	Passage Number	[MTX]	Freezing Date	Location (in box)	Tested	Amplification
1	CHO TNFγ 1.2	112995-P06	20nM	01.30.96	A1-3	AV	+
2	CHO TNFγ 1.3	112995-P06	20nM	01.30.96	B1-3	AV	+
3	CHO TNFγ 1.7	112995-P06	20nM	01.30.96	C1-3	AV	+
4	CHO TNFγ 1.9	112995-P06	20nM	01.30.96	D1-3	AV	+
5	CHO TNFγ 2.7	112995-P06	20nM	01.30.96	E1-3	AV	+
6	CHO TNFγ 1.6	112995-P07	20nM	02.01.96	F1-3	AV	+
7	CHO TNFγ 6.4	112995-P04	20nM	02.01.96	G1-3	AV	+
8	CHO TNFγ 6.7	112995-P04	20nM	02.01.96	H1-3	AV	+
9	CHO TNFγ 6.8	112995-P04	20nM	02.01.96	A4-6	AV	+
10	CHO TNFγ 2.3	112995-P06	20nM	02.02.96	B4-6	AV	+
11	CHO TNFγ 2.9	112995-P06	20nM	02.02.96	C4-6	AV	+
12	CHO TNFγ 3.3	112995-P05	20nM	02.02.96	D4-6		
13	CHO TNFγ 6.2	112995-P04	20nM	02.02.96	E4-6		
14	CHO TNFγ 6.6	112995-P04	20nM	02.02.96	F4-6		
15	CHO TNFγ 6.10	112995-P04	20nM	02.02.96	G4-6		
16	CHO TNFγ 1.10	112995-P05	20nM	02.05.96	H4-6		
17	CHO TNFγ 5.5	112995-P04	20nM	02.05.96	A8-10		
18	CHO TNFγ 5.9	112995-P04	20nM	02.05.96	B8-10		
19							
20							
22							
23							
24							
25							

Immunoprecipitation with TNFγ - Mouse - Serum

Western with TNFγ - PPT - Mouse Serum Ref Ellie Barfield



Samples that were Immuno Precipitated:

1.) CHOSFM II + 1% dFBS
2.) ST 10.1 / 011696 - P03
3.) CHOTNFγ 1.2
4.) CHOTNFγ 1.3
5.) CHOTNFγ 1.7
6.) CHOTNFγ 1.9
7.) CHOTNFγ 2.7
8.) CHOTNFγ 1.6
9.) CHOTNFγ 6.4
10.) CHOTNFγ 6.7

1.) CHO TNFα 6.8
2.) CHO TNFα 2.3
3.) CHO TNFα 2.9

1.) dilute mouse anti-hTNF 1:10 (2 µl in 18 µl PBS) (T175)
2.) add 1 µl of the 1:10 AB dilution to 500 µl CHO cell supernatant
3.) incubate 30', RT, on rotator
4.) wash Protein G (Gamma bind Plus Sepharose Pharmacia Code No. 17-0886-01)
- spin 1 ml of Protein G, 5 sec at 10000 rpm
- aspirate sup, wash with PBS
- repeat 1x
- resuspend Protein G in PBS + 0.1% BSA
5) add 20 µl Protein G to sample
6) incubate for 30' RT, rotator
7) Spin 10-20 sec. at 10K RPM
- aspirate Supernatants
8) wash 3x with 1 ml PBS
- Spin, remove supernatant
9) add 20 µl 2x Sample buffer, boil 10'
- spin after boiling
10.) Transfer supernatants into new Eppendorf tube and freeze at −20°C

(→ load samples on gel)

29.96 Comparative study ST5, ST10.1

take 3 x 1 ml aliquots from each clone and passage in MEM α, 5% dFBS, 1% PS, 80 μM MTX
Split 1:3

1) 346 ST5 / 011196 p07 (2 T75 flasks)
2) CHO ST10.1 / 011696 p07 (2 T75 flasks)

29.96 CHO dhfr- -DG44

CHO dhfr- / DG44 / 010996 - P13 Split 1:8

passage into MEM α, 5% dFBS, 1% PS

29.96 Establishing of stable 346-VIGF5 cells

amplification up to 80 μM MTX:
(continued from P52)

cells grown in 6 wells at 50 μM, 80 μM, 100 μM MTX

- pool cells growing at the concentrations 50 μM, 80 μM, 100 μM and seed into a T75 flask in 80 μM MTX (MEM α, 5% dFBS, 1% PS)

29.96 Establishing of stable CHO TNFR clones.

freezing of backup in process cells:

1.) CHOTNFR 5-7 / 112995 - p05
2.) CHOTNFR 5-4 / 112995 - p04
3.) CHOTNFR 4-2 / 112995 - p05
4.) CHOTNFR 6-5 / 112995 - p04
5.) CHOTNFR 4-1 / 112995 - p05

Establishing stable 346 TNFR clones:

expanding of backup clones: (for freezing)

6well: seed: 53

24well: seed: 54

- replace the medium with MEM1, 5% dFBS, 1% PS, 20 mM HEPES

Establishing of stable 346 TNFR clones:

Split confluent T25 flasks:

50 %: seed in T25 flask in MEM1, 5% dFBS, 1% PS, 20 mM HEPES for freezing

25 %: seed in T25 flask in MEM1, 5% dFBS, 1% PS, 20 mM HEPES for testing, replace medium with CHO-S-SFM-II + 1% Sem + 1% PS tomorrow.

25 %: seed in 6 wells of a 6well dish for amplification MTX concentrations:
0, 50, 100, 150, 250, 500 nM MTX
(→ 1 µM-MTX stock solution!)

1.) 346 TNFR 2.3 / 112995 - pos
2.) 346 TNFR 4.1 / 112995 - pos
3.) 346 TNFR 4.3 / 112995 - pos
4.) 346 TNFR 4.4 / 112995 - pos
5.) 346 TNFR 4.5 / 112995 - pos
6.) 346 TNFR 4.6 / 112995 - pos
7.) 346 TNFR 4.7 / 112995 - pos
8.) 346 TNFR 4.10 / 112995 - pos
9.) 346 TNFR 5.3 / 112995 - pos
10.) 346 TNFR 5.4 / 112995 - pos

Establishing stable CHO-TNFR clones:

29.9.96 Proteingel and Electrotransfer for Western Assays:

(continued from pi. 63)

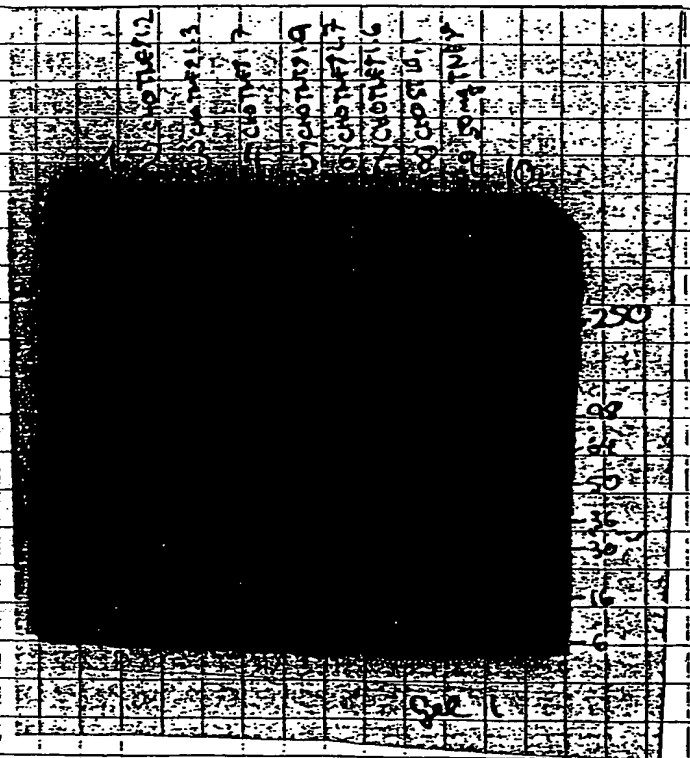
- the supernatants were concentrated by immunoprecipitation and prepared like described on p. 60 - 63!
- ran 2 proteingels: 4-20 % NOVEX
  68 mA
  1 h

Gel 1: 1.) M (NOVEX prest. LWM)
2.) 1.2 CHO TNFR
3.) 1.3 -"-
4.) 1.7 -"-
5.) 1.9 -"-
6.) 2.7 -"-
7.) 1.6 -"-
8.) neg control (LST 10.1)
9.) pos control 50ng TNFR
10.) M

Gel 2: 1.) M
2.) 6.4 CHO TNFR
3.) 6.7 -"-
4.) 6.8 -"-
5.) 2.3 -"-
6.) 2.9 -"-
7.) pos control 50ng TNFR
8.) M
9.) M
10.) neg control (CHO-S-SFNT)

Western blot : Novex Nitrocellulose Membrane 2.12.96

- precast in Bottling buffer
- electrotransfer at 30V ; 1h
- 1h blocking 4% nonfat milk
- 2 x 10' wash with 1 x PBS
- 2.5h incubation with 1st AB (Mouse-anti TNFR-PPT AB dil. 1:5000 + T.175 see p. 62)
- rinse 3x with 1 x PBS, wash 3 x 10' with 1 x PBS
- 1h incubation with 2nd AB 1:2000 (anti Mouse AB HRP)
- rinse 3 x with 1 x PBS, wash 3 x 10' with 1 x PBS
- 1' detection ECL
- 1 sec. exposure



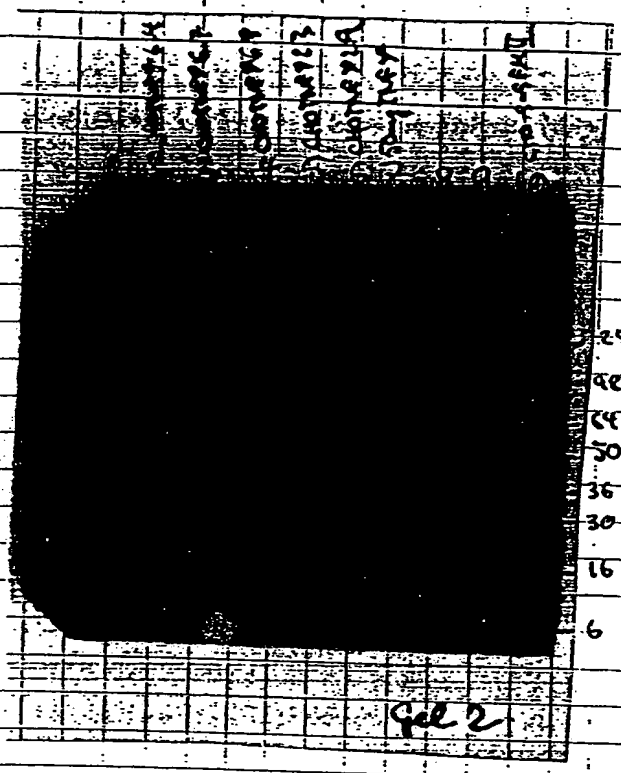


fig 2

2.12.96 Establishing of stable 346 TNFα clones

Change Medium to CHO-S-SFM II + 1% PS of the cells in T25 flasks for testing (see p. 65)

- remove the TCM
- wash 2 x with PBS
- add 5 ml of CHO-S-SFM II + 1% PS to each T25 flask

1.) 346 TNFα 2.3 / 112995 p03
2.) 346 TNFα 4.1 / 112995 p03
3.) 346 TNFα 4.3 / 112995 p03
4.) 346 TNFα 4.4 / 112995 p03
5.) 346 TNFα 4.5 / 112995 p03
6.) 346 TNFα 4.6 / 112995 p03
7.) 346 TNFα 4.7 / 112995 p03
8.) 346 TNFα 4.10 / 112995 p03
9.) 346 TNFα 5.3 / 112995 p03
10.) 346 TNFα 5.9 / 112995 p03

CHO clone DG44/010996 p03-14 2.12.96

- passage cells: split ratio 1:10 and prepare cells f. transfection
- resuspend 3 ml of the cell susp. in 11.5 ml MEM α^-, 5% dFBS, 1% PS
- seed 1 ml of this cell suspension into each of 12 wells of 2 6-well dishes (1.45 ml)

→ cells for transfection

Comparative Study 346 STS - CHO ST10.1 2.12.96

take 3 x 1 ml aliquots from each clone and passagem MEM α^-, 5% dFBS, 1% PS, 80 nM MTX

1.) 346 STS/011696 p08 (split ratio 1:4)
2.) CHO ST10.1/011696 p08 (split ratio 1:2)

Establishing of stable 346 TNFR clones and CHO TNFR clone 2.13.96

freezing of in process cells after clonal selection, expanding of clones into T 75 flasks (see p. 65)

1.) 346 TNFR 2.3 / 112995 p03
2.) 346 TNFR 4.1 / 112995 p03
3.) 346 TNFR 4.3 / 112995 p03
4.) 346 TNFR 4.4 / 112995 p03
5.) 346 TNFR 4.5 / 112995 p03
6.) 346 TNFR 4.6 / 112995 p03
7.) 346 TNFR 4.7 / 112995 p03
8.) 346 TNFR 4.10 / 112995 p03
9.) 346 TNFR 5.3 / 112995 p03
10.) 346 TNFR 5.4 / 112995 p03
11.) CHO TNFR 4.3 / 112995 p04
12.) CHO TNFR 4.4 / 112995 p04

2.14.96 Establishing stable TNFγ clones
(346TNFγ, derived from pU346 vector)

- harvested TCM from T25 flasks (SFM + 1% PS) for testing in Western analysis and antiviral assays

1.)	346TNFγ 2.3 / 112995 p03	(5 × 1ml)
2.)	346TNFγ 4.1 / 112995 p03	(5 × 1ml)
3.)	346TNFγ 4.3 / 112995 p03	(5 × 1ml)
4.)	346TNFγ 4.4 / 112995 p03	(5 × 1ml)
5.)	346TNFγ 4.5 / 112995 p03	(5 × 1ml)
6.)	346TNFγ 4.6 / 112995 p03	(5 × 1ml)
7.)	346TNFγ 4.7 / 112995 p03	(3 × 1ml)
8.)	346TNFγ 4.10 / 112995 p03	(5 × 1ml)
9.)	346TNFγ 5.3 / 112995 p03	(4 × 1ml)
10.)	346TNFγ 5.4 / 112995 p03	(4 × 1ml)

- harvested 3, 4 or 5 × 1ml aliquots
- submitted 2 × 1ml aliquots of each clone to Dan Becknell for testing in antiviral assays (except clone 346TNFγ 4.7 submitted only 1 × ml)
- freeze remaining 1ml aliquots for testing in western blots

2.14.96 Establishing stable TNFγ clones (346TNFγ derived from pU346)

- expanding of backup clones from 24 well or 24 well plates into T25 flasks
  → expand cells for freezing of backup clones in MEM + 20mM HEPES + 5% DMSO + 1% PS from 6 well dishes:

1.)	346TNFγ 4.2 / 112995 p03
2.)	346TNFγ 4.8 / 112995 p03
3.)	346TNFγ 4.9 / 112995 p03
4.)	346TNFγ 5.1 / 112995 p03
5.)	346TNFγ 5.2 / 112995 p03

from 24 well plates:

6.) 346TNFX 6.1 / p02 CM
7.) 346.TNFX 6.2 / p02 V
8.) 346.TNFX 6.3 / p02
9.) 346.TNFX 6.4 / p02 CM
10.) 346TNFX 6.5 / p02 X
4.) 346TNFX 6.6 / p02
12.) 346.TNFX 6.7 / p02
13.) 346TNFX 6.8 / p02
14.) 346TNFX 6.9 / p02
15.) 346TNFX 6.10 / p02

Establishing of stable CHO TNFX clones: 2.15.96

Amplification: continued from p 58

cells growing at 0-1000nM MTX pooled and seeded at 0, 100, 200, 500, 800 and 1000nM MTX

1.) CHO TNFX 1.2 / 112995 p08
2.) CHO TNFX 1.3 / 112995 p08
3.) CHO TNFX 1.7 / 112995 p08
4.) CHO TNFX 1.9 / 112995 p08
5.) CHO TNFX 2.2 / 112995 p08

cells growing at 0-400nM pooled and seeded at 0, 50, 100, 150, 250, 500 nM MTX

1.) CHO TNFX 1.6 / 112995 p07
2.) CHO TNFX 2.3 / 112995 p07
3.) CHO TNFX 2.9 / 112995 p07
4.) CHO TNFX 6.8 / 112995 p08

5.) CHO TNFX 6.9 / 112995 p06 } exchange TCM in
6.) CHO TNFX 6.2 / 112995 p06 } dead cells, same MTX like other clones (1-4)

2.15.96 Establishing of stable 346 TNFβ clones

Amplification continued from p 65

cells grown in wells from 0-500nM MTX
pool cells and seed in 6 wells at a 6 well dish
at 0, 50, 100, 150, 250 and 500nM MTX

(1μM MTX stock solution)

1.) 346 TNFβ 2.3 / 112995 p04
2) 346 TNFβ 4.1 / 112995 p04
3) 346 TNFβ 4.3 / 112995 p04
4) 346 TNFβ 4.4 / 112995 p04
5.) 346 TNFβ 4.5 / 112995 p04
6.) 346 TNFβ 4.6 / 112995 p04
7.) 346 TNFβ 4.7 / 112995 p04
8.) 346 TNFβ 4.10 / 112998 p04
9.) 346 TNFβ 5.3 / 112995 p04
10.) 346 TNFβ 5.4 / 112995 p04

2.16.96 CHO after DG44 passaging of cells

CHO after DG44 / 010996 p15 split ratio 1:10
in MEM α, 5% dFBS, 1% PS

Establishing of stable 346 VIGFS clone

amplification up to 100μM MTX
continued from p. 64

- remove medium in T25 flask and replace with
fresh MEM α, 5% dFBS, 1% PS and 80μM MTX

Comparative study 346 STS, CHO ST 10.1 — 12.16.96

take 3 x 1 ml aliquots of each clone and passage cells in MEM-, 5% dFBS, 1% PS, 80 µM MTX

1.) 346 STS / 011696 p09
2) CHO ST10.1 / 011696 p09

12.20.96

Comparative study 346 STS, CHO ST 10.1

take 3 x 1 ml aliquots of each clone and passage cells in MEM-, 5% dFBS, 1% PS, 80 µM MTX

1.) 346 STS / 011696 p10
2) CHO ST10.1 / 011696 p10

Establishing of stable 346 VCAM5 clone

- amplification up to 80 µM MTX
- passage cells and seed in 2 T25 flasks for freezing and analysis of cells in MEM-, 5% dFBS, 1% PS, 80 µM MTX

Establishing of stable 346 TNFγ clones (continued p. 20)

- expanding of backup clones for freezing passage cells from T25 flasks into T75 flasks in MEM-, 5% dFBS, 1% PS, 20 nM MTX

1.) 346 TNFγ 4.2 / 112995 p04
2.) 346 TNFγ 4.8 / 112995 p04
3.) 346 TNFγ 4.9 / 112995 p04
4.) 346 TNFγ 5.1 / 112995 p04
5.) 346 TNFγ 5.2 / 112995 p04
6.) 346 TNFγ 6.3 / 112995 p03
7.) 346 TNFγ 6.6 / 112995 p03
8.) 346 TNFγ 6.7 / 112995 p03
9.) 346 TNFγ 6.8 / 112995 p03
10.) 346 TNFγ 6.9 / 112995 p03
11.) 346 TNFγ 6.10 / 112995 p03

2.20.96 – for two of the 346 TNFγ clones the medium was replaced by MEMα, 5% dFBS, 1% PS, 20 nM MTX

1.) 346 TNFγ 6.1 / U2995 p 02
2.) 346 TNFγ 6.4 / U2995 p 02

Passaging of CHO^{dhfr-} DG44 cells

1.) passage cells in T 225 flasks CHO^{dhfr-} DG44 / 010996 p16 split ratio 1:8 in MEMα, 5% dFBS, 1% PS (2.20.96)

2.) resuspend remaining cells in a final volume of 10 ml

resuspend 3 ml of this cellsuspension in 13.5 ml final volume

label 1 ml of this dilution to each of the wells of 2-6 well dishes for transfection

Establishing of stable CHO TNFγ cell lines

duplication of clones: continued p. 71

cells growing at 0 – 1000 nM MTX, pool cells in several wells and seed at 0, 100, 200, 500, 800 and 1000 nM MTX

1.) CHO TNFγ 1.2 / U2995 p02
2.) CHO TNFγ 1.3 / U2995 p09
3.) CHO TNFγ 1.7 / U2995 p09
4.) CHO TNFγ 1.9 / U2995 p09
5.) CHO TNFγ 2.7 / U2995 p09

cells growing at 0-500 nM MTX, pooled and seeded at 0, 100, 200, 500, 800 and 1000 nM MTX

1.) CHOTNFX 1.6 / 112995 p 08
2.) CHOTNFX 2.3 / 112995 p 08
3.) CHOTNFX 2.9 / 112995 p 08
4.) CHOTNFX 6.8 / 112995 p 06
5.) CHOTNFX 6.4 / 112995 p 07
6.) CHOTNFX 6.7 / 112995 p 07

Establishing of new stable CHO-cell lines 2.21.96

Transfection of CHO dhfr- DG44 cells with expression vectors:

1.) CESP / HHFHG78 / ProjectCode HG11600 / pC1 (Dan Sappet)
2.) GDF / HITER36 / ProjectCode HG11000 / pC1 (Dan Sappet)
3.) VEGF2 / HOSBD47 / ProjectCode HG00400 / pC1 (Jing-Shan Hu)
4.) FGF-11 / HE9CC44 / ProjectCode HG07000 / pC1 (Jing-Shan Hu)
5.) Control pN18 only
6.) control no DNA added to cells

- CHO dhfr- DG44 / DG44 / 010996 p16 cells were seeded for transfections in 96 well ata 6 well dish the day before
- cells were 70-80 % confluent
- remove TCM, wash cells 1x with PBS, remove PBS
- add 1 ml of OptiMEM + 1 % FCS to each well
- prepare for each transfection reaction in 96 well ata 96 well dish

A: 90 µl OptiMEM
10 µl Lipofectin

B: 50 µl OptiMEM, 5 µg Expression vector, 0.5 µg pN18

- Mix A and B and incubate at RT for 45'
- Add Transfection mix to the cells and incubate for 5h at 37°C, 5% CO_2.
- Add 1ml of Opti MEM + 0% dFBS + 1% PS

2.22.96 Establishing of CHO-TNFδ clones and 346-TNFδ clones

Initial screening for TNFδ expressing clones by Western Analysis

- Sample preparations: all TNFδ clones were incubated for 2 days in CHO-S-SFM-II + 1% PS before the medium was harvested and frozen.
  see p. 48 and p56 for CHOTNFδ clones
  see p 70 for 346TNFδ clones

- 11 x CHO-TNFδ clones, that in parallel are amplified and, 10 x 346-TNFδ clones were tested for TNFδ expression in Western Assays
- samples of each clone were loaded on a 16% NOVEX Gel
  a) 30µl medium straight
  b) 500µl medium TCA precipitated

TCA - Precipitation:

- 500 µl supernatant
  + 50 µl 0.15% DOC
  + 70 µl 100% TCA

- vortex 5' on ice
- 5' 14000 RPM
- remove sup. add 100 µl acetone
- 2' on ice
- 5' 14000 RPM

- resuspend pellet in 30 µl 0.1 N NaOH + 0.1% SDS
- add 10 µl 4 X SDS loading buffer

positive controls: 1.) samples that were not precipitated: (straight)
50ng E. coli expressed and purified (Mark Oel) ~~TNF γ was added to 30 µl of CHO-S-SFM-II + 10 µl sample buffer~~

2.) precipitated samples: (precip.)
50 ng TNFγ was added to 500 µl CHO-S-SFM-II and the TCA precipit. was done as described above

II CHOTNFγ clones: all gels 16% NOVEX gels

Gel 1:
1) M
2) CHOTNFγ 1.2 30 µl straight
3) CHOTNFγ 1.3 -"-
4) CHOTNFγ 1.7 -"-
5) CHOTNFγ 1.9 -"-
6) CHOTNFγ 2.7 -"-
7) CHOTNFγ 1.6 -"-
8) CHO ST10.1 -"-
9) 50ng TNFγ straight
10) M

Gel 2:
1) M
2) CHOTNFγ 6.4 30 µl straight
3) CHOTNFγ 6.7 -"-
4) CHOTNFγ 6.8 -"-
5) CHOTNFγ 2.3 -"-
6) CHOTNFγ 2.9 -"-
7) ~~[illegible]~~ 50ng TNFγ straight
8) CHO ST10.1 30 µl straight
9) M
10) M

I CHOTNFγ clones:

Gel 3:

1.) M
2.) CHOTNFγ 1.2 ~~30µl straight~~ 500µl precipitated
3.) CHOTNFγ 1.3 —11—
4.) CHOTNFγ 1.7 —11—
5.) CHOTNFγ 1.9 —11—
6.) CHOTNFγ 2.7 —11—
7.) CHOTNFγ 1.6 —11—
8.) CHO ST 10.1 —11—
9.) 50ng TNFγ 30µl straight
10.) M

Gel 4:

1.) M
2.) CHOTNFγ 6.4 500µl precip.
3.) CHOTNFγ 6.7 500µl precip.
4.) CHOTNFγ 6.8 500µl precip.
5.) CHOTNFγ 2.3 500µl precip.
6.) CHOTNFγ 2.9 500µl precip.
7.) CHO ST 10.1 500µl precip.
8.) 50ng TNFγ straight
9.) 50ng TNF precipitated
10.) M

II 346 TNFγ samples:

Gel 1:

1.) M
2.) 346TNFγ 2.3 30µl straight
3.) 346TNFγ 4.1 30µl straight
4.) 346TNFγ 4.3 30µl straight
5.) 346TNFγ 4.4 30µl straight
6.) 346 TNFγ 4.5 30µl straight
7.) CHO ST.10.1 30µl straight
8.) 50ng TNFγ straight
9.) M
10.) M

Gel 2:

1.) M
2.) 346TNFγ 4.6 30µl straight
3.) 346TNFγ 4.7 30µl straight
4.) 346TNFγ 4.10 30µl straight
5.) 346TNFγ 5.3 30µl straight
6.) 346TNFγ 5.4 30µl straight
7.) ~~CHO ST 10.1~~ CHO ST 10.1 30µl st.
8.) ~~50ng TNFγ straight~~ 50ng TNFγ straight
9.) M
10.) M

2:22.96 UB

II 346 TNF γ samples:

Gel 3:

1.) M
2.) 346 TNFγ 2.3 500 μl precipit.
3.) 346 TNFγ 4.1 500 μl precipit.
4.) 346 TNFγ 4.3 500 μl precipit.
5.) 346 TNFγ 4.4 500 μl precipit.
6.) 346 TNFγ 4.5 500 μl precipit.
7.) CHO ST 10.1 500 μl precipit.
8.) 50 ng TNFγ straight
9.) 50 ng TNFγ precipit.
10.) M

Gel 4:

1.) M
2.) 346 TNFγ 4.6 500 μl precip.
3.) 346 TNFγ 4.7 500 μl precip.
4.) 346 TNFγ 4.10 500 μl precip.
5.) 346 TNFγ 5.3 500 μl precip.
6.) 346 TNFγ 5.4 500 μl precip.
7.) CHO ST 10.1 500 μl precip.
8.) 50 ng TNFγ straight
9.) 50 ng TNFγ precip.
10.) M

Western blot: Novex Nitrocellulose Membrane

- presoak memb. in blotting buffer
- electrotransfer at 30 V 1h
- 1h blocking at 4°C, 4% milk, rinse + wash memb.
- 2.5 incubation with 1st AB

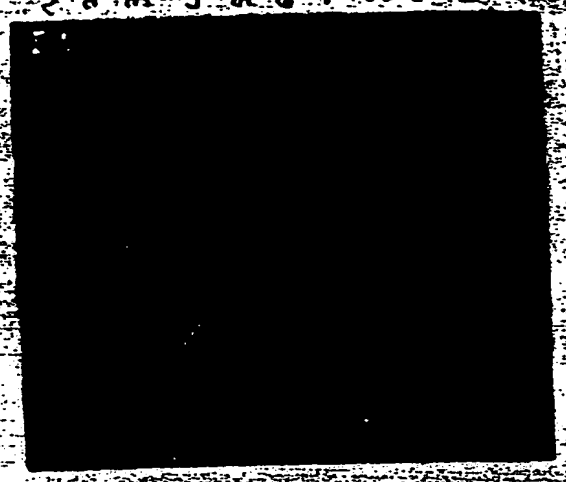
T173 (see p. 62) 1:5000

- rinse 2× with 1×PBS and wash 3× with 1×PBS, 10'
- 1h incubation with 2nd AB 1:2000 (anti-Mouse AB)

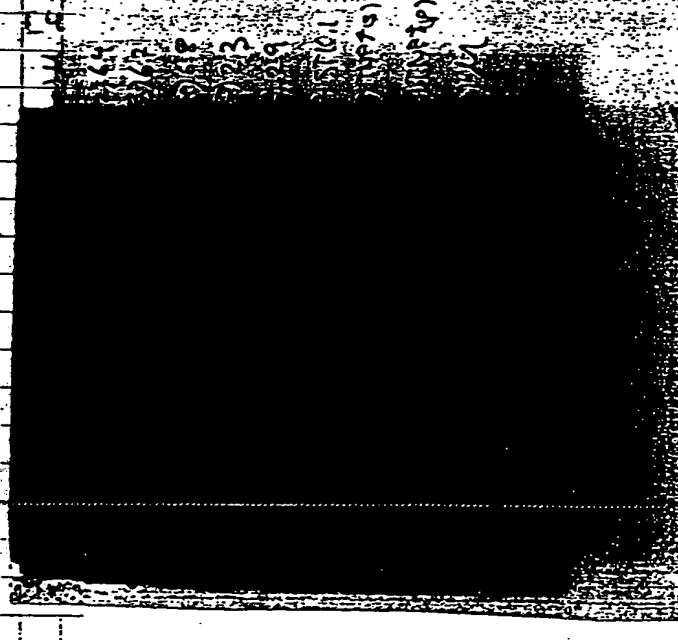
- Result Western: W. needs dose optimized, Transfection[illegible]

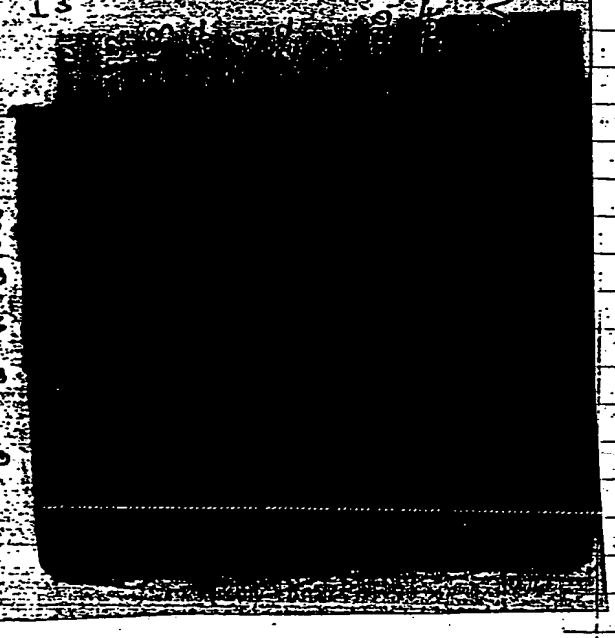
1.) - Background reduction, use TWEEN-PBS
2.) - first AB incubation over night
3.) - antibody specificity: AB was derived from E. coli expressed
- material, precipit.

CHO TNF-2 clones (112995 pK) Western first screening
(samples: 25µl TCA)

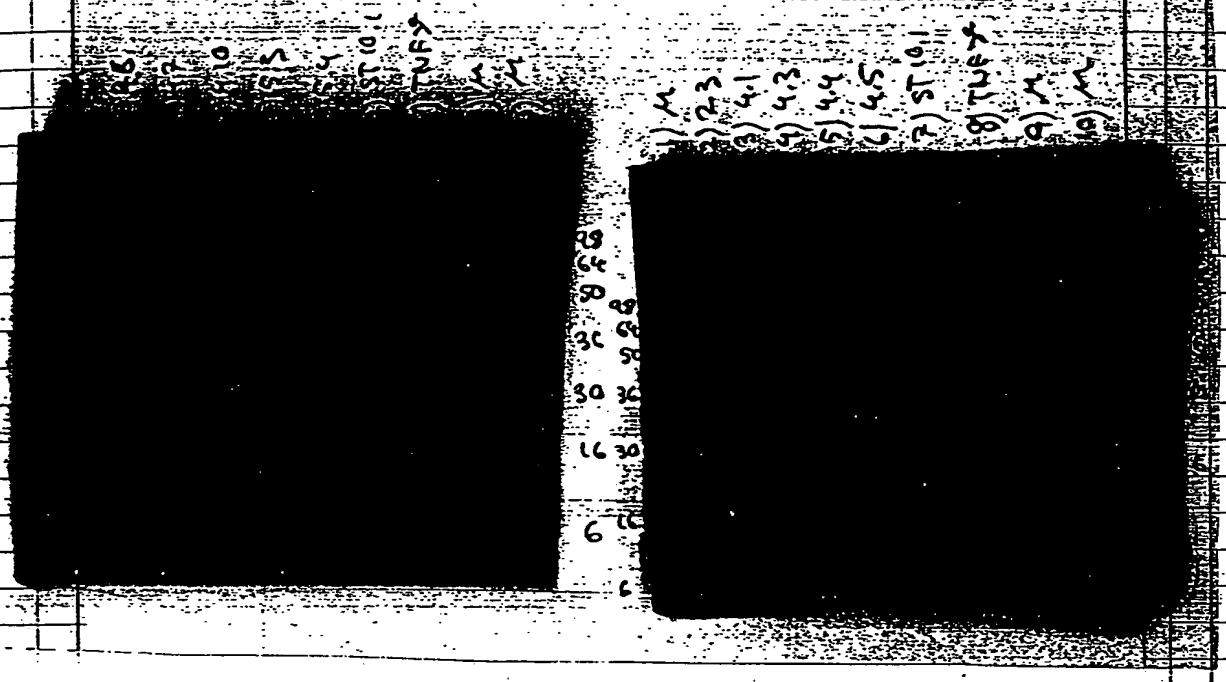


CHO TNF-2 clones (112995 pK) Western first screening
samples: 500µl TCA TCA-precip.)

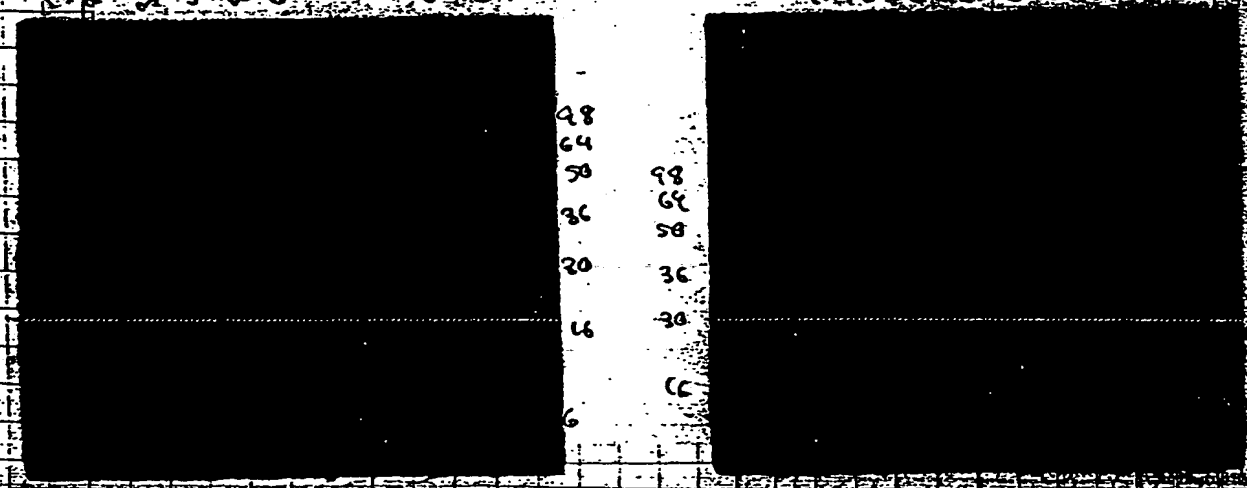




346 TNF δ clones / 112995 pX / Western first screening
(samples 80μl TCM supernat)



CHO TNFδ clones / 112995 pX Western first screening



2.23.96 Establishing of stable CHO clones:

CESP, GDF, VEGF2, FGF 11

continued from P. 75

Selection of recombinant CHO-clones for amplification

- Cells that were transfected in 6well dishes (see p. 75) were seeded in 3 96well dishes each clone in MEMα, 5% dFBS, 1% PS, 10nM MTX, (mg/ml G418.

96well plates:

1.) CESP-1 / 022196 p01
2.) CESP-2 / 022196 p01
3.) CESP-3 / 022196 p01

4.) GDF-1 / 022196 p01
5.) GDF-2 / 022196 p01
6.) GDF-3 / 022196 p01

7.) VEGF2-1 / 022196 p01
8.) VEGF2-2 / 022196 p01
9.) VEGF2-3 / 022196 p01

10.) FGF-11-1 / 022196 p01
11.) FGF-11-2 / 022196 p01
12.) FGF-11-3 / 022196 p01

13.) negative control N18-1
14.) -"- N18-2
15.) -"- N18-3

16.) -"- -1 (no DNA)
17.) -"- -2 (no DNA)
18.) -"- -3 (no DNA)

CHO cells DG44/ 2.23.96

CHO cells DG44/ 010996 p14 split 1:8
passage into MEMα, 5% dFBS, 1% PS

SUPERVISOR- Junlin Zhang
DATE- 2/23/96

Comparative Study 346 STS, CHO ST10.1 2.23.96

take 3x 1ml aliquots of each clone and passage cells split 1:3 in Memα, 5% dFBS, 1% PS, 80% RH

1.) 346 STS 011696 p11 (freeze also 2.29.96)
2.) CHO ST10.1/011696 p11 (")

Establishing of stable 346 VIGFS clone
- amplification up to 80% RH
- passage cells and seed in 2 x T25 flasks for freezing and analysis

Establishing of stable CHO clones 346 TNFR 2.29.96
(continued from p. 73)

freezing of backup clones

1.) 346 TNFR 6.8 p.03
2.) 346 TNFR 6.9 p.03
3.) 346 TNFR 6.10 p03
4.) 346 TNFR 4.9 p04
5.) 346 TNFR 4.8 p04
6.) 346 TNFR 4.2 / 112995 p 04
7.) 346 TNFR 4.7 / 112995 p.03
8.) 346 TNFR 6.3 / 112995 p 03
9.) 346 TNFR 6.6 / 112995 p 03
10.) 346 TNFR 5.11 / 112998 p 04
11.) 346 TNFR 5.2 / 112998 p 04

2.24.96 Establishing stage, CHO TNFγ cell lines

Amplification: continued from p 74, 75

cells were growing at 100 – 1000 nM MTX
pools confluent wells at 800 nM and 1000 nM and seed in 0; 0,5; 1; 1,5; 3 and 5 µM MTX.

1.) CHO TNFγ 2,3 / 112995 p09
2.) CHO TNFγ 2.7 / 112995 p10
3.) CHO TNFγ 4.3 / 112995 p10
4.) CHO TNFγ 6.4 / 112995 p8
5.) CHO TNFγ 6.7 / 112995 p08

2.26.96 Comparative Study ST5, ST 10.1

take 3 x 1 ml aliquots each clone and passage cells 1:4 in MEM α, 5% dFBS, 1% PS, 80 µM MTX

1.) 346 ST5 / 011696 p 12
2) CHO ST10.1 / 011696 p 12

CHO dhfr⁻ / DG44

CHO dhfr⁻ / DG44 / 010996 p 15 Split 1:10
MEM α, 5% dFBS, 1% PS,

5mM MTX Stock solution:

- dissolve 4 100mg MTX in 44 ml MEM α - Medium
- filtersterilize through a 0.2 µm filter
- aliquot in 14 x 1 ml aliquots and 6 x 5 ml aliquots

Establishing of stable 3T TNFγ clones 2.27.96

Amplification continued from p 72

- cells grow at low density, many dead cells in suspension
- remove supernatant and wash each well with 2 ml PBS
- Add 2 ml of MEMα, 5% dFBS, 1% PS to each well to recover cells at all different wells

Establishing stable CHO TNFγ clones 2.29.96

continued p. 84 Amplification

cells were growing at concentrations between 0 and 5 μM MTX

1.) poole cells at all concentrations in 7 ml final volume

a) - seed 1 ml in each well of a 6 well dish at 0; 0,5; 1; 1.5; 3 and 5 μM MTX

b) - seed 1 ml into a T75 flask in MEMα* and expand for testing (baseline etc)

1.) CHO TNFγ 2.3 / 112995 p10 *
2.) CHO TNFγ 2.7 / 112995 p10
3.) CHO TNFγ 1.3 / 112995 p10
4.) CHO TNFγ 6.4 / 112995 p09
5.) CHO TNFγ 6.7 / 112995 p09

2.27.96 MP

2.2.96 Establishing of stable CHO TNFβ clones

continued from p 74 Amplification

1.) - cells are going at 0-1000 nM MTX
- dead cells in suspension cells 40-50% confluent
- wash with PBS and passage in 6well dishes at 0-1000 nM MTX

1) CHO TNFγ 1.6 / 112995 p09
2) CHO TNFγ 6.9 / 112995 p07
3) CHO TNFγ 1.2 / 112995 p10
4) CHO TNFγ 1.9 / 112995 p10

2.) wash with PBS and replace TCM (~~same~~ MTX)

1.) CHO TNFβ 1.7 / 112995 p09
2.) CHO TNFβ 2.9 / 112995 p08

3.1.96 Establishing stable 346 TNFγ cell lines

continued from p 85

Amplification: cells that grew at MTX concentr. from 50 to 200 nM before were going in MEM - medium without MTX to recover and expand the cells (see p. 85)
The cells were pooled and seeded in 0, 100, 200, 500, 800 and 1000 nM MTX

1.) 346TNFγ 2.3 / 112995 p05
2.) 346TNFγ 4.1 / 112995 p05
3.) 346TNFγ 4.3 / 112995 p05
4.) 346TNFγ 4.4 / 112995 p05
5.) 346TNFγ 4.5 / 112995 p05
6.) 346TNFγ 4.6 / 112995 p05
7.) 346TNFγ 4.7 / 112995 p05
8.) 346TNFγ 4.10 / 112995 p05

2.) 346 TNFR 5.3 / 112995 pos
10.) 346 TNFR 5.4 / 112995 pos

Comparative Study STS, ST 10.1

take 3 x 1ml aliquots each clone and passage cells 1:3 in Mem L, 5% dFBS, 1% PS, 80,000 MTX

1.) 346 STS / 011696 p13
2.) EHO ST 10.1 / 011696 p13

Amplification of VIGF 5:

from p13 - passage cells in MEM L, 5% dFBS, 1% PS 80,000 MTX
(for freezing and labeling)

Comparative Study of several VIGF and TNFR clones (Labeling with ^{35}S-Cys + ^{35}Met)

Trial clones:	freezing date:	
1.) STS (100,000 MTX)	10.5.95	STS / 030696 p01
2.) VIGF5 (5,000 MTX)	12.7.95	VIGF5 / 030196 p01
3.) VIGF3 (100,000 MTX)	12.7.95	VIGF3 / 030196 p01

seed cells in T25 at 80,000 resp. 0,0 MTX

CHO dhfr- DG44 / 010996 p16 split 1:10
Mem L F, 5% dFBS, 1% PS

3.1.96 MP

VIGF3 received from Alenia seeded in T75 for labeling:

VIGF3 p15

Labeling of ST, VIGF and TNF8 cell lines

3.4.96 Prepare cells for labeling experiment:

- cells were seeded in T75 flasks before. (see previous pages)

- for labeling experiment seed cells into the wells of a 6 well dish in MEMα^+, 5% dFBS, 1% PS

	T75 flask	dish in 6 well
1.) 346 ST5 / 032196 p02	70%	1:7
2.) VIGF3 (5 µM) / 030196 p02	100%	1:10
3.) VIGF3 (100 µM) /	80%	1:8
4.) VIGF3 / 030196 p02	80%	1:8
5.) VIGF3 / p16	100%	1:10
6.) CHOTNF8 2.7 / 112995 p12	20%	1:2
7.) CHOTNF8 4.3 / 112995 p12	30%	1:3
8.) CHOTNF8 6.4 / 112995 p10	50%	1:5
9.) CHOTNF8 6.7 / 112995 p10	70%	1:7

Comparative study ST5, ST10.1

- take 3x 1ml aliquots of each clone and passage cells 1:4 in MEMα^-, 5% dFBS, 1% PS, 80 µM MTX

346 ST5 / 041696 p14
CHO ST10.1 / 041696 p14

Establishing stable 346 TNFγ cell lines

9.5.96

continued from p. 86

cells growing from 0 - 1000 pM MTX. cells are confluent at 800 pM and 1 pM
pool cells at 800 nM + 1 µM and seed into 6 wells of a 6 well at 0/0.5/1/1.5/3 and 5 µM.

1.) 346 TNFγ / 112995 p06 / 2.3
2.) 346 TNFγ / 112995 p06 / 4.1
3.) 346 TNFγ / 112995 p06 / 4.3
4.) 346 TNFγ / 112995 p06 / 4.4
5.) 346 TNFγ / 112995 p06 / 4.5
6.) 346 TNFγ / 112995 p06 / 4.6
7.) 346 TNFγ / 112995 p06 / 4.7
8.) 346 TNFγ / 112995 p06 / 4.10
9.) 346 TNFγ / 112995 p06 / 5.3
10.) 346 TNFγ / 112995 p06 / 5.4

Establishing stable CHO TNFγ cell lines

3.5.96

continued from p. 86

cells growing up to 1 µM MTX

pool cells at different concentrations and seed in 0/0.5/1/1.5/3 and 5 µM MTX.

1.) CHO TNFγ 1.7 / 112995 p10
2.) CHO TNFγ 6.8 / 112995 p08
3.) CHO TNFγ 2.9 / 112995 p09

3.5.96 UB

3.5.96 Labeling of ST, VIGF and TNFγ cell lines

1.) 346 ST.5
2.) VIGFS (5μM)
3.) VIGFS (10μM MTX)
4.) VIGF3 / 030196 p02
5.) VIGF3 / p16
6.) CHO TNFγ 2.7 / 112995 p 12
7.) CHO TNFγ 4.3 / 112995 p 12
8.) CHO TNFγ 6.4 / 112995 p10
9.) CHO TNFγ 6.7 / 112995 p10
10.)

cells are centrifuged in 6well dish
add to each well:

add: ^{35}S - Cysteine and ^{35}S-Methionine
5μl of each AA to each well (10μCi/μl)

3.6.96 Establishing of recombinat CHO TNFγ clones

continued from P. 85: Amplification

cells are growing at 0-5μM MTX
Pools cells are established in 6 wells at
0 / 0.5 / 1 / 1.5 / 3 and 5μM MTX

1.) CHO TNFγ 2.3 / 112995 p11 (pools all wells)
2.) CHO TNFγ 2.7 / 112995 p12 (pools 1μM - 5μM MTX
3.) CHO TNFγ 4.3 / 112995 p12 —11—
4.) CHO TNFγ 6.4 / 112998 p10 —11—
5.) CHO TNFγ 6.7 / 112998 p10 (pools 3μM + 5μM)

Testing:

For testing in the anticirus assay cells of the following clones were seeded into 6 T25 flasks in MEM-, 0 mM, 5% dFBS, 1% PS:

1.) CHO TNFR 2.2 / 112995 p 12 (0.5 µM MTX)
2.) CHO TNFR 4.3 / 112995 p 12 (0.5 µM MTX)
3.) CHO TNFR 6.4 / 112995 p 10 (0.5 µM MTX)
4.) CHO TNFR 6.2 / 112995 p 10

3.6.95

Establishing of these CHO TNFR clones:

continued from p 86:

cells were growing at 0 - 1000 nM MTX before:

1) cells that were growing at 300 nM 500 nM and 1 µM were pooled and seeded in 0 / 500 / 1000 / 1500 / 3000 and 5000 nM MTX

1.) CHOTNFR 1.6 / 112996 p 10
2.) CHOTNFR 1.9 / 112996 p 4
3.) CHOTNFR 1.2 / 112996 p 4

2) Prepare cells for testing:

cells growing at 200 nM MTX were seeded into a T25 flask in MEM-, 0 MTX, 5% dFBS, 1% PS for testing in the anticiral assay:

1.) CHO TNFR 1.6 / 112996 p 10
2) CHO TNFR 1.9 / 112996 p 4
3.) CHO TNFR 1.2 / 112996 p 4

S.A. 96 MB

3.11.96 Establishing of stable CHO TNFγ cell lines

continued from p. [illegible], [illegible], 89, 90, 91

Amplification

1.)	CHO TNFγ 1.2 / 112995 p 12
2.)	CHO TNFγ 1.6 / 112995 p 11
3.)	CHO TNFγ 1.7 / 112995 p 11
4.)	CHO TNFγ 1.9 / 112995 p 12
5.)	CHO TNFγ 2.3 / 112995 p 12
6.)	CHO TNFγ 2.7 / 112995 p 13
7.)	CHO TNFγ 2.9 / 112995 p 10
* 8.)	CHO TNFγ 4.3 / 112995 p 13
* 9.)	CHO TNFγ 6.4 / 112995 p 11
* 10.)	CHO TNFγ 6.7 / 112995 p 11
* 11.)	CHO TNFγ 6.8 / 112995 p 09

cells were growing in 6 wells 0 - 5 µM MTX
pools cells of each clone and seed in
0,05, 1, 1.5, 3 and 5 µM MTX

Establishing stable 346 TNFα cell lines 3.12.96

continued from p. 89

1.) Harvest supernatants for artificial assay (Dan Redundant)

2.) pool cells and seed at 0-5 μM MTX (2nd time these counts.)

1.) Harvest for testing: (see p. 89)

1.) 346 TNFα 2.3 / poc
2.) 346 TNFα 4.1 / poc
3.) 346 TNFα 4.3 / poc
4.) 346 TNFα 4.4 / poc
5.) 346 TNFα 4.5 / poc
6.) 346 TNFα 4.6 / poc
7.) 346 TNFα 4.7 / poc
8.) 346 TNFα 4.10 / poc
9) 346 TNFα 5.3 / poc
10) 346 TNFα 5.4 / poc

(cells were growing at 0-1 μM MTX before seeded in selection MTX)

cells each clone growing in 6 well at different MTX concentr. (0-5 μM)

harvest sup of each clone growing at 0 MTX and submit for artificial assay.

2.) seed cells in new 6 well dish for amplification:

1.) 346 TNFα 2.3 / 112995 poz
2.) 346 TNFα 4.1 / 112995 poz
3.) 346 TNFα 4.3 / 112995 poz
4.) 346 TNFα 4.4 / 112995 poz
5.) 346 TNFα 4.5 / 112995 poz

6.) 396 TNFγ 4.6 / 112995 p 87
7.) 396 TNFγ 4.7 / 112995 p 87
8.) 396 TNFγ 4.10 / 112995 p 87
9.) 396 TNFγ 5.3 / 112995 p 87
10.) 396 TNFγ 5.4 / 112995 p 87

cells growing at different concentrations were pooled and seeded at 0, 0.5, 1, 1.5, 3 and 5 μM MTX

3.12.96 CHO TNFγ samples submitted for antiviral assay (Dan Bechanik)

continued from p. 91

clones were growing in T25 flasks in Mem α⁻, 0 MTX, 5% dFBS, 1% PS

			seeded from:
1.) CHOTNFγ 1.2 / 112995	p11		0.2 μM MTX
2.) CHOTNFγ 1.6 / 112995	p10		0.2 μM MTX
3.) CHOTNFγ 1.9 / 112995	p11		0.2 μM MTX
4.) CHOTNFγ 2.7 / 112995	p12		0.5 μM MTX
* 5.) CHOTNFγ 4.3 / 112995	p12		0.5 μM MTX
6.) CHOTNFγ 6.4 / 112995	p10		0.8 μM MTX
7.) CHOTNFγ 6.7 / 112995	p10		0.8 μM MTX

* clone CHO TNFγ 1.3 was mislabeled on tube that was submitted to Dane, tube label: CHO TNFγ 4.3

3.12.96 [illegible]

Comparative Study ST5, ST10.1 3.13.96

from p.94:

harvest 3 × 1 ml aliquot of each clone and freeze at -80°C.

Passage cells 1:3 in 2 T75 flasks each clone at MEMα, 5% dFBS, 1% PS, 80 μM MTX

1.) 346 ST5 / 011696 p16
2.) CHO ST10.1 / 011696 p16

Amplification of VIGFS up to 100 μM MTX:

from p. 87:

- passage cells 1:3 in T75 flask in MEMα, 5% dFBS 1% PS, 80 μM MTX.

VIGFS / 031396 p01

Freezing of ST5, ST10.1 and VIGFS 3.13.96
clones from above:

freeze 3 × 1 ml aliquots 10^6 cells of

1.) 346 ST5 / 011696 p15 at 80 μM MTX
2.) CHO ST10.1 / 011696 p15 at 80 μM MTX
3.) VIGFS / 031396 p00 at 80 μM MTX

(protocol see p50)

3.13.96 [initials]

3.13.96 Establishing of FGF11 clones:

continued from p 82 Project Code: HE 07000
HE9CC44

passaging of 48 clones from Hybridoma plate cells into 24 wells into Ham F, 5% dFBS, 1% PS, 10nM MTX
see P. 92

FGF11 - 1/022196 p02 up to FGF11-48/022196p02

3.14.96 Establishing of stable CHO TNF8 cell lines

Continued from p. 96

Amplification:

- Cells confluent in 5 μM MTX:
- pool cells in 3 μM MTX and 5 μM MTX
- seed 50 % of the pooled cells in 6 wells of a 6 well dish in 0, 2.5, 5, 7.5, 12.5 and 25 μM MTX
- first seeded at these concentrations

1.) CHO TNF8 1.3 // 112996 p 14
2.) CHO TNF8 6.4 // 112996 p 12
3.) CHO TNF8 6.7 // 112996 p 12
4.) CHO TNF8 6.8 // 112996 p 18